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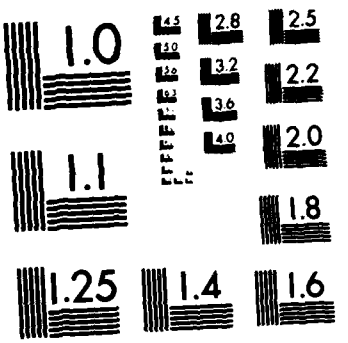

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UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010

PHASE 1
DERMAL PENETRATION AND DISTRIBUTION OF ^{14}C-LABELED PARANITROPHENOL (PNP)
STUDY NO. 75-51-0047-84
FEBRUARY - APRIL 1983

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
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7. AUTHOR(s) Hubert L. Snodgrass, Jr.		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Commander US Army Enviromental Hygiene Agency Aberdeen Proving Ground, MD 21010		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS Commander US Army Health Services Command Ft Sam Houston, TX 78234		12. REPORT DATE February - April 1983 13. NUMBER OF PAGES 13
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18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Absorption	Health Hazard	Tissues
Animals	Leather Treatment	
Dermal	Paranitrophenol	
Distribution	p-Nitrophenol	
Excretion	Radiolabeled	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The skin absorption potential of paranitrophenol, a leather fungicide, was assessed in rabbits and dogs. Absorption of the topically applied radiochemical measured 35 percent of the applied dose in rabbits and 11 percent in dogs through 7 days. No affinity for tissue binding was demonstrated. Based on this study and others, it is estimated that 10 percent or less of PNP reaching the skin surface of man would be absorbed. Leather footwear treated with PNP at 0.35 percent is not expected to represent a health hazard.

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DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

Mr. Snodgrass/dlc/AUTOVON
584-3980

REPLY TO ATTENTION OF

HSHB-OT/WP

31 OCT 1983

SUBJECT: Phase 1, Dermal Penetration and Distribution of ^{14}C-Labeled Paranitrophenol (PNP), Study No. 75-51-0047-84, February - April 1983

HQDA (DASG-PSP)
Washington, DC 20310

EXECUTIVE SUMMARY

The purpose, essential findings and conclusions of the inclosed report follow:

a. Purpose. Paranitrophenol (PNP) is a leather fungicide used to treat combat boots and other leather commodities supplied to military personnel. The skin absorption potential of PNP, its bodily distribution and elimination, was assessed in rabbits and dogs using the ^{14}C-labeled chemical.

b. Essential Findings. Absorption of topically applied PNP measured 35 percent of the applied dose in rabbits and 11 percent in dogs through 7 days. The chemical was rapidly metabolized in the animal body and eliminated by urinary excretion. Enteric elimination was negligible. Disappearance of PNP from circulating dog blood (half-life) occurred within 15 minutes of an intravenous injection. No affinity for tissue binding of PNP moieties was observed in either animal model.

c. Conclusions. Based upon observations in animals and earlier tests performed at this Agency, it is estimated that 10 percent or less of PNP reaching the skin surface of man would be absorbed. Leather footwear treated with PNP at 0.35 percent is not expected to represent a health hazard.

FOR THE COMMANDER:

1 Incl
as

for Rodney D. Richards
JOEL C. GAYDOS, M.D.
Colonel, MC
Director, Occupational and Environmental Health

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DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

REPLY TO ATTENTION OF

HSHB-OT/WP

PHASE I
DERMAL PENETRATION AND DISTRIBUTION OF ^{14}C-LABELED PARANITROPHENOL (PNP)
STUDY NO. 75-51-0047-84
FEBRUARY - APRIL 1983

1. AUTHORITY. Letter, DRXNM-ZT, 31 August 1977, subject: Toxicological Studies Required for Registration of Paranitrophenol as a Leather Fungicide, and indorsements thereto.

2. REFERENCES. See Appendix A for a listing of references.

3. PURPOSE. The purpose of this study was to quantitate the rate of penetration of ^{14}C-labeled PNP through the intact skin of rabbits and dogs. The dermal absorption and bodily distribution of PNP was assessed by monitoring the radioactivity in excreta for 7 days and monitoring selected tissues at necropsy. Kinetics of PNP following parenteral administration were also determined. The methodology has been previously described (reference 2).

4. GENERAL.

a. See Appendix B for the Bibliography.

b. See Appendix C for Tables.

c. In conducting the studies described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," US Department of Health, Education and Welfare Publication No. (NIH) 78-23, revised 1978.

d. The studies reported herein were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care. This report and data generated in this study are stored in Toxicology's file located in Room 3011, Building E2100, APG-EA 21010.

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e. See Appendix D for information on Analytical Quality Assurance.

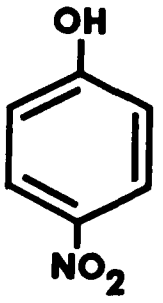
5. BACKGROUND. Paranitrophenol (PNP) is a leather fungicide used in various commodities supplied to Army personnel. Combat boots present the highest potential for dermal exposure. About 2.5 g of PNP is incorporated into each pair. Although the chemical currently holds conditional use approval by the US Environmental Protection Agency (File NO. 40510-E), further subchronic testing is required before final registration. Paranitrophenol has been selected by the National Cancer Institute (NCI number C55992) for bioassay and chronic testing is now in progress. Metabolism studies are concurrently indicated (reference 1, Appendix A). The rabbit has been suggested for dermal tests (reference 1, Appendix A) because of its tendency towards maximum absorption potential. The beagle dog has also shown promise as an animal model for dermal testing because it more closely resembles man's absorption kinetics.[1]

6. MATERIALS.

a. Radiolabeled PNP [ring - $^{14}C(U)$] was custom synthesized by New England Nuclear, Boston, Massachusetts 02118. It was identified as Lot No. 1135-045 and contained a reported radiochemical purity of 98.5 percent, as determined by thin layer chromatography and radiochromatogram. Specific activity was 3.9 millicuries/millimole and total mass of 357 mg.

b. Absolute (100 percent) ethanol was the vehicle used in all dilutions of PNP for animal administration.

c. The chemical structure of PNP is as follows:



7. ANIMALS.

a. Six male New Zealand White rabbits, weighing between 2.5 and 3.3 kg, were purchased from Dutchland Laboratories, Denver, Pennsylvania. Animals were individually housed in Wahmann stainless steel metabolism cages, and received food (Aberdeen 09 Rabbit Ration, Zeigler Bros, Garners, Pennsylvania) and water ad libitum.

b. Six male purebred Beagle dogs, 25-months old, were selected from USAEHA kennel stock. These animals were originally purchased from Laboratory Research Enterprises, Inc., Kalamazoo, Michigan. Dogs were housed individually in Wahmann stainless steel metabolism cages and received food (Respond 2000, ProPet, Inc., Syracuse, New York) and water ad libitum.

8. METHODS.

a. Three rabbits received a single intravenous injection of ^{14}C-labeled PNP to assure that systemic elimination of the chemical was measurable in excreted urine. Each animal received a radioactive dose of 10 microcuries (μCi) and a chemical mass of 357 micrograms (μg) injected into the marginal ear vein. The 0.25 ml volume was introduced into a saline infusion (2.5 mL) to prevent the necrotizing effect of the alcohol on the vein lumen.

b. Three rabbits received the chemical topically. The mid-lumbar area of the back was clipped free of hair and the application area demarcated with petrolatum to contain the chemical within the predetermined 8.9 cm^2 area. The applied dose was the same as described in paragraph 8a, this report. The application rate was 40.1 $\mu g/cm^2$ for each animal. The application area was then covered with a nonocclusive patch which protected the area without contacting the radiochemical. The patch was changed at 24 hours and a new one affixed.

c. Dogs also received PNP as a single intravenous or topical dose. Three dogs were treated intravenously with a 0.5 mL volume containing 20 μCi of radioactivity and 714 μg of PNP. Injection was made into the cephalic vein via a saline infusion. The remaining three dogs received the same dose topically contained in a 17.8 cm^2 area or application rate of 40.1 $\mu g/cm^2$. The area was covered with a nonocclusive patch and changed at 24 hours.

d. Dogs receiving intravenous PNP were monitored at timed intervals to assess disappearance of radiocarbon from circulating blood. A semilog plot of radioactivity in blood versus time was constructed using the "stripping" technique.[2] The half-life ($t_{1/2}$) was determined for the rapid disappearance phase.

e. Excreta was collected and measured at 24-hour intervals through the 7-day test period. Aliquots (0.2 mL) of urine were combined with 15 mL of PCS●II scintillation cocktail and radioactivity measured using a Beckman Model LS 9000, Beckman Liquid Scintillation Counter. Internal standardization techniques and automatic quench correction procedures were employed. Feces were collected daily, weighed, and combined with 2 volumes of methanol. After mixing for 24 hours, aliquots (0.2 mL) of the supernate were combined with PCS II and counted.

f. At the end of the study period, animals were euthanized and representative tissue and fluid specimens collected and measured for radiocarbon content. Specimens included liver, lung, kidney, spleen, heart, brain and adrenal gland. Also collected were urinary bladder, muscle, bone, skin, fat, thyroid gland, testes, bone marrow, blood and bile. Radioactivity was assessed following oxidation of each 0.25 - 0.45 g specimen to $^{14}CO_2$ using a Hewlett-Packard Biological Materials Oxidizer. Blood specimens were centrifuged and 0.2 mL aliquots of plasma added to PCS II for counting.

g. Unabsorbed PNP from topically treated animals was quantitated by extracting the nonocclusive patches and the excised skin from the application site in methanol. Extract fractions (0.2 mL) were combined with the scintillation cocktail and counted.

h. Excretion rates of radiocarbon were calculated as the percent recovery each day of the injected or applied dose appearing in urine or feces. Calculations for tissue specimens collected at necropsy were based on counts (cpm) per specimen and reported as dpm/g of wet tissue following correction for counting efficiency.

9. RESULTS.

a. Urinary excretion accounted for essentially all of the eliminated ^{14}C-labeled PNP following a single intravenous injection in rabbits and dogs. Total radioactivity recovered in urine through 7 days measured 79 percent in rabbits and 95 percent in dogs (Table C-1, Appendix C). Over 96 percent of the eliminated radiocarbon was recovered within the first 24 hours after injection in both species. Radioactivity in feces through the 7-day test measured 1 percent or less of the injected dose in rabbits and dogs. Tissue specimens collected at necropsy from both species did not contain detectable ^{14}C-labeled chemical except two skin specimens from dogs which measured less than 5 ppb of PNP.

●PCS is a registered tradename of Amersham Corp, Arlington Heights, Illinois. Use of trademarked names does not imply endorsement by the US Army, but is intended only to assist in the identification of a specific product.

b. The disappearance of ^{14}C-labeled PNP from the circulating blood in dogs, following a single intravenous injection, indicated a half-life of about 15 minutes. Circulating radiocarbon was nearly undetectable 4 hours after injection.

c. Recovery of absorbed ^{14}C-labeled PNP following topical application to rabbits is shown in Table C-2, Appendix C. Absorption measured 35 percent of the applied dose through 7 days as measured by urinary excretion. Seventy-eight percent of PNP recovered in urine appeared within day 1 and was essentially complete by day 4. Fecal elimination accounted for less than 1 percent of the applied dose. Unabsorbed PNP recovered at the site of application and from the patches, accounted for 53 percent of the applied chemical. Tissue specimens collected at necropsy contained no detectable radiocarbon.

d. Absorption of ^{14}C-labeled PNP in dogs totaled 11 percent of the topical dose as measured in urine through 7 days (see Table C-3, Appendix C). An absorption rate of 3 percent/day was observed through the first 48 hours and declined to less than 1 percent/day by day 5. Fecal elimination was negligible, measuring less than 0.5 percent of the applied dose for the entire test period. Eighty-six percent of the applied dose remained unabsorbed at 7 days. Tissue specimens from dogs contained no detectable radioactivity except for two specimens from one animal; both contained less than 5 ppb of PNP.

10. DISCUSSION.

a. The PNP is rapidly metabolized and eliminated from the animal body. Elimination is by urinary excretion which may account for all endogenous chemical. Though minor amounts of labeled PNP moieties were detected in feces, they in fact may be contributed through urine contamination.

b. It has been shown that in rabbits, PNP metabolism involves conjugation with glucuronic (60-80 percent) and sulfonic acid (10-20 percent) and reduction to aminophenols (10 percent); less than 1 percent of the dose is excreted unchanged as nitrophenols.[3] While the acid conjugates are excreted rapidly, reduction to aminophenols may take 48 hours.[3] The observed absence of labeled PNP in tissue specimens from dogs and rabbits supports an efficient metabolic clearance.

c. The potential for human exposure to PNP treated leather is inherent to military personnel wearing treated low quarter shoes or combat boots. An average pair of treated combat boots contains about 2.5 g of PNP (720 g of dry leather and treatment rate of 0.35 percent PNP)(see reference 3, Appendix A). Based upon a 10 percent absorption potential as demonstrated in dogs, 250 mg could be absorbed by a man during the expected life of the boots. This, of course, is highly unlikely as it assumes intimate contact between leather and skin and total leaching of PNP out of the leather.

d. Socks probably afford the greatest protection from treated footwear. It has been reported that while significant leaching from leather does occur into stocking material (7 day wear test), transfer to the skin surface was not observed (see reference 3, Appendix A). Apparently, the stocking material traps leached PNP from leather. Since the subjects changed socks daily, it is difficult to assess the effects of a cumulative buildup of chemical in the stockings.

e. In another study, dermatologic reactions were monitored in 115 subjects wearing low quarter shoes overtreated (1.1 percent) with PNP (see reference 4, Appendix A). No reactions were attributed to PNP leather treatment through 5 weeks of wear. The investigators concluded that the overtreated shoes, under conditions of normal wear, did not represent a health hazard.

11. CONCLUSIONS. Based upon observations in animals and earlier tests performed at this Agency, the following conclusions can be offered:

a. Fractional absorption of PNP occurs following skin contact. On entry to the systemic circulation, the chemical is rapidly metabolized and excreted in the urine. No potential for chemical binding in animal tissue has been demonstrated.

b. It is estimated that 10 percent or less of PNP reaching the skin surface of man would be absorbed.

c. Military footwear (leather) treated with PNP at 0.35 percent is not expected to represent a health hazard.

HUBERT L. SNODGRASS, JR.
Biologist
Toxicology Division

APPROVED:

MAURICE H. WEEKS
Chief, Toxicology Division

APPENDIX A

REFERENCES

1. Proposed guidelines for Registering Pesticides in the United States; Hazard Evaluation: Humans and Domestic Animals, 43 Federal Register (FR) 37336, 22 August 1978.

2. USAEHA, Toxicology Division Standing Operating Procedure, Radioisotope Studies, February 1983.

3. Letter, DRDNA-VCC, US Army Natick Research and Development Laboratories, 23 January 1981, subject: Concentration of p-Nitrophenol in Combat Boots.

4. Memorandum For The Record, USAEHA-MM, US Army Environmental Hygiene Agency, 2 February 1967, subject: High PNP Content of Shoes (Gardiner Shoe Company).

5 Letter, HSE-LT/WP, this Agency, 21 August 1980, subject: Protocol, Reproduction Study, Paranitrophenol Study No. 75-51-0047-80.

APPENDIX B

BIBLIOGRAPHY

1. Snodgrass, H.L., D.C. Nelson and M. H. Weeks, "Dermal Penetration and Potential for Placental Transfer of the Insect Repellent N, N-Diethyl-m-toluamide," Am Ind Hyg Assoc J, 43 (10), 747-753 (1982).

2. Wagner, J.G., "Linear Compartment Models," Fundamentals of Clinical Pharmacokinetics, pp 57-62, Drug Intelligence Publications, Hamilton, Illinois (1975).

3. Robinson, D., J.N. Smith and R.T. Williams, Biochem J, 221 (1951a), as cited in Detoxification Mechanisms, 2nd ed. R.T. William. d. John Wiley and Sons, Inc., New York, New York (1959).

APPENDIX C

TABLES

TABLE C-1. ELIMINATION OF ^{14}C-LABELED PNP FOLLOWING A SINGLE INTRAVENOUS INJECTION TO RABBITS AND DOGS.

RABBIT(3)
MEAN PERCENT OF INJECTED DOSE

DAY	URINARY EXCRETION	FECAL EXCRETION
1	78.51 ± 10.50	0.18 ± 0.10
2	0.56 ± 0.18	0.03 ± 0.01
3	0.10 ± 0.02	0.03 ± 0.01
4	0.08 ± 0.02	0.05 ± 0.01
5	0.08 ± 0.05	0.02 ± 0.01
6	0.01 ± 0.00	0.05 ± 0.02
7	0.06 ± 0.04	0.04 ± 0.01
TOTAL	79.40 ± 10.76	0.40 ± 0.10

DOG(3)
MEAN PERCENT OF INJECTED DOSE

DAY	URINARY EXCRETION	FECAL EXCRETION
1	91.66 ± 9.35	0.68 ± 0.75
2	1.09 ± 0.62	0.09 ± 0.02
3	0.86 ± 0.76	0.09 ± 0.05
4	0.77 ± 0.80	0.06 ± 0.02
5	0.37 ± 0.39	0.04 ± 0.00
6	0.32 ± 0.35	0.04 ± 0.00
7	0.21 ± 0.22	0.02 ± 0.00
TOTAL	95.28 ± 10.03	1.02 ± 0.84

Study No. 75-51-0047-84, Feb - Apr 83

TABLE C-2. FATE OF ^{14}C-LABELED PNP FOLLOWING A SINGLE TOPICAL APPLICATION TO RABBITS (2).

MEAN PERCENT OF APPLIED DOSE

DAY	URINARY EXCRETION	FECAL EXCRETION
1	27.24 ± 5.02	0.14 ± 0.03
2	4.88 ± 1.99	0.06 ± 0.03
3	1.73 ± 0.64	0.05 ± 0.04
4	0.59 ± 0.16	0.08 ± 0.02
5	0.30 ± 0.16	0.06 ± 0.00
6	0.24 ± 0.15	0.07 ± 0.03
7	0.08 ± 0.04	0.07 ± 0.04
TOTAL	35.11 ± 1.90	0.53 ± 0.16

TOTAL PERCENT RECOVERY

		APPLICATION	PATCHES		
URINE	FECES	SITE	DAY 1	DAY 7	TOTAL
35.11 ±1.90	0.53 ±0.16	31.82 ±1.70	4.59 ±1.17	17.07 ±6.41	89.12 ±5.04

NOTE:
One rabbit topically treated with PNP ingested some of the protective patch. The data was omitted due to the artificially high excretion of radioactivity.

Study No. 75-51-0047-84, Feb - Apr 83

TABLE C-3. FATE OF ^{14}C-LABELED PNP FOLLOWING A SINGLE TOPICAL APPLICATION TO DOGS (3).

MEAN PERCENT OF APPLIED DOSE

DAY	URINARY EXCRETION	FECAL EXCRETION
1	3.04 ± 1.57	0.07 ± 0.02
2	3.96 ± 0.96	0.03 ± 0.01
3	1.57 ± 0.00	0.04 ± 0.01
4	1.11 ± 0.25	0.06 ± 0.02
5	0.83 ± 0.27	0.09 ± 0.01
6	0.67 ± 0.19	0.07 ± 0.05
7	0.21 ± 0.16	0.05 ± 0.00
TOTAL	11.39 ± 1.52	0.41 ± 0.11

TOTAL PERCENT RECOVERY

		APPLICATION	PATCHES		
URINE	FECES	SITE	DAY 1	DAY 7	TOTAL
11.39 ±1.52	0.41 ±0.11	38.76 ±10.83	14.90 ±11.58	32.63 ±6.40	98.09 ±3.38

APPENDIX D

ANALYTICAL QUALITY ASSURANCE

The Analytical Quality Assurance Office certifies the following with regard to study 75-51-0047-83, Dermal Penetration and Distribution of ^{14}C-Labeled Paranitrophenol (PNP):

a. This study was conducted in accordance with

(1) Standing Operating Procedures developed by the Toxicology Division, USAEHA.

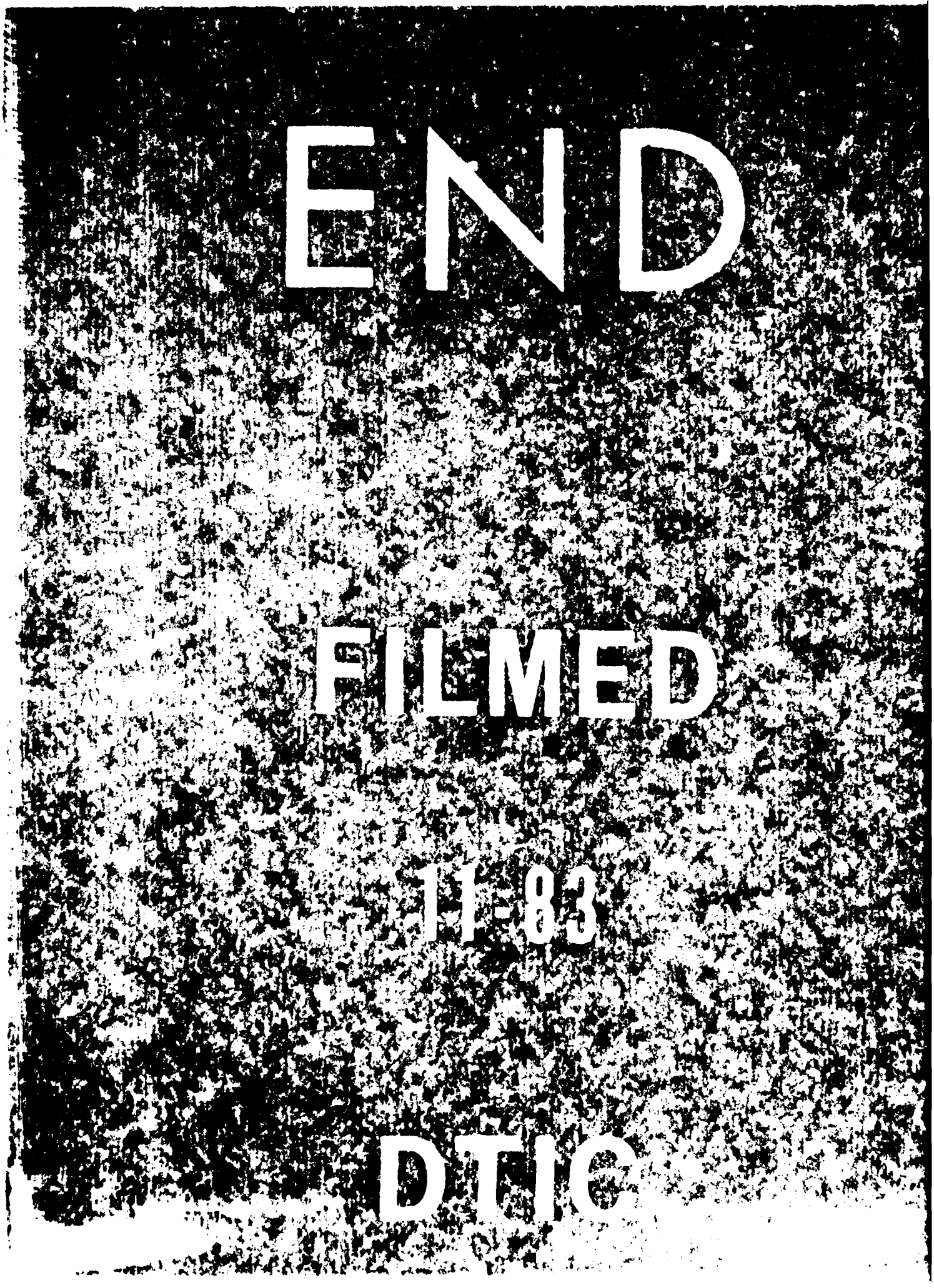
(2) Proposed guidelines for Registering Pesticides in the United States; Hazard Evaluation: Humans and Domestic Animals, 43 Federal Register (FR) 37336, 22 August 1978.

b. Facilities were inspected on the following dates during its operational phase to insure compliance with paragraph a above:

Dogs	Rabbits
24 February 1983	15 March 1983
3 March 1983	23 March 1983
14 March 1983	14 October 1983
14 October 1983	

c. The information presented in this report accurately reflects the raw data generated during the course of conducting the study.

PAUL V. SNEERINGER, Ph.D.
Chief, Analytical Quality
Assurance Office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